

Serial No.: 09/600,392

Confirmation No.: 4850

Filed: September 8, 2000

For: AN AUTOREGULATORY SYSTEM FOR VALIDATING MICROBIAL GENES AS POSSIBLE
ANTIMICROBIAL TARGETS USING A TETRACYCLINE-CONTROLLABLE ELEMENT

Sub SC
1. [AMENDED] A process for the identification of a microbial gene encoding a gene product that is important to a microbe's ability to infect or sustain an infection in a mammal, which process comprises:

infecting a plurality of mammals with a microbe that has been genetically altered such that the amount of said gene product produced by said genetically altered microbe is regulated by a Tetracycline-Controllable Element (TCE),

where said TCE is a gene regulatory system that controls the expression of the target gene product through its ability to modulate the function of said gene in response to said microbe's exposure to tetracycline, and where said TCE is comprised of a tetracycline-controllable transcription promoter polynucleotide sequence;

where said genetically altered microbe also comprises a polynucleotide sequence encoding a tetracycline resistance protein;

exposing the plurality of mammals to tetracycline;

once an infection with the genetically altered microbe is established, removing the tetracycline exposure of a portion of the plurality of mammals, such that a first group of the plurality of mammals is exposed to tetracycline and a second group of the plurality of mammals is not exposed to tetracycline; and

comparing the degree of infection, microbe levels, or survival rates of the mammals in the first group and the second group wherein a meaningful difference between the two groups of animals identifies the gene product as important to a microbe's ability to infect or sustain an infection in a mammal.

2. [AMENDED] The process of claim 1, where said TCE is operably linked to a polynucleotide sequence encoding a reporter gene (RG).

3. [AMENDED] The process of claim 1, where said tetracycline-controllable transcription promoter polynucleotide sequence is a prokaryotic transcription promoter.

Serial No.: 09/600,392

Confirmation No.: 4850

Filed: September 8, 2000

For: AN AUTOREGULATORY SYSTEM FOR VALIDATING MICROBIAL GENES AS POSSIBLE ANTIMICROBIAL TARGETS USING A TETRACYCLINE-CONTROLLABLE ELEMENT

4. [AMENDED] The process of claim 1, where said TCE is operably linked to a polynucleotide sequence encoding a reporter gene (RG) and a target gene (TG).

5. [AMENDED] The process of claim 4, where said reporter gene encodes a β -lactamase.

6. [AMENDED] The process of claim 1, where said polynucleotide sequence encoding a tetracycline resistance protein is contained on a tetracycline resistance and repressor DNA cassette (TRRDC), said TRRDC comprising a tetracycline repressor gene and a tetracycline resistance gene.

7. [AMENDED] The process of claim 6, where said TCE is operably linked to a polynucleotide sequence encoding a reporter gene (RG) and a target gene (TG) and where the TCE, the TRRDC, the RG, and the TG are all on the same DNA cassette, referred to as a Regulatory DNA Cassette (RDC).

8. [AMENDED] The process of claim 6, where said TRRDC promoter is operably linked to the TCE, the tetracycline repressor gene comprises the structural gene *tetM*, and the tetracycline resistance gene comprises the structural gene *tetR*.

9. [AMENDED] The process of claim 1, where said meaningful difference between the two groups of animals is a meaningful difference in the levels of microbes or levels of infection present in the mammals.

10. [AMENDED] The process of claim 1, where said meaningful difference between the two groups of animals is a meaningful difference in the survival rates of the groups of animals.

Serial No.: 09/600,392

Confirmation No.: 4850

Filed: September 8, 2000

For: AN AUTOREGULATORY SYSTEM FOR VALIDATING MICROBIAL GENES AS POSSIBLE
ANTIMICROBIAL TARGETS USING A TETRACYCLINE-CONTROLLABLE ELEMENT

11. [AMENDED] The process of claim 1, where said meaningful difference between the
two groups of animals shows that animals exposed to tetracycline have poorer health, higher
rates of infection, lower survival or higher levels of microbes than animals not exposed to
tetracycline.

*CD
cel*

12. [AMENDED] The process of claim 6, where said tetracycline resistance gene of said
TRRDC comprises sequences from the *Staphylococcus aureus tetM* gene.

13. [AMENDED] The process of claim 6, where said tetracycline repressor gene of said
TRRDC is obtained from the Tn10 transposon.

14. [AMENDED] The process of claim 6, where said TRRDC comprises the sequence of
SEQ ID NO:35 or SEQ ID NO:36.

B

15. [AMENDED] The process of claim 1, where said infected mammals are mice.

16. [AMENDED] The process of claim 1, where said genetically altered microbe is a
Staphylococcus species.

17. [AMENDED] The process of claim 16, where said *Staphylococcus* species is
Staphylococcus aureus.

18. [AMENDED] The process of claim 1, where said microbe is a virus.

19. [AMENDED] The process of claim 1, where said microbe is a lower eukaryote.

20. [AMENDED] The process of claim 1, where said microbe is a yeast.

Serial No.: 09/600,392

Confirmation No.: 4850

Filed: September 8, 2000

For: AN AUTOREGULATORY SYSTEM FOR VALIDATING MICROBIAL GENES AS POSSIBLE
ANTIMICROBIAL TARGETS USING A TETRACYCLINE-CONTROLLABLE ELEMENT

77. [AMENDED] A process to regulate expression of a gene product by a microbe in a mammalian host with tetracycline or a tetracycline analog, said process comprising:

 infecting a mammalian host with a microbe that has been genetically altered such that the amount of said gene product produced by said genetically altered microbe is regulated by a Tetracycline-Controllable Element (TCE);

B2
 where said TCE is a gene regulatory system that controls the expression of the target gene product through its ability to modulate the function of said gene in response to said microbe's exposure to tetracycline, and where said TCE is comprised of a tetracycline-controllable transcription promoter polynucleotide sequence;

 where said genetically altered microbe also comprises a polynucleotide sequence encoding a tetracycline resistance protein; and

 exposing the mammalian host to tetracycline.

78. [NEW] The process of claim 77, further comprising, once an infection with the genetically altered microbe is established, removing the tetracycline exposure of the mammalian host.

B3
79. [NEW] The process of claim 1, where said plurality of mammals are exposed to tetracycline while being infected with the genetically altered microbe.

80. [NEW] The process of claim 1, where said plurality of mammals are exposed to tetracycline by adding tetracycline to the drinking water.

81. [NEW] The process of claim 2, where said reporter gene encodes a β -lactamase.